ELSEVIER

Biochimica et Biophysica Acta 1428 (1999) 29–38

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

New biodegradable hydrogels based on a photocrosslinkable modified polyaspartamide: synthesis and characterization

Gaetano Giammona [a,*], Giovanna Pitarresi [a], Gennara Cavallaro [a], Silvestre Buscemi [b], Filippo Saiano [c]

[a] *Dipartimento di Chimica e Tecnologie Farmaceutiche, Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy*
[b] *Dipartimento di Chimica Organica 'E. Paternò', Università degli Studi di Palermo, Viale delle Scienze, 90128 Palermo, Italy*
[c] *Dipartimento di Ingegneria e Tecnologie Agro-Forestali, Università degli Studi di Palermo, Viale delle Scienze, 90128 Palermo, Italy*

Received 8 January 1999; received in revised form 25 March 1999; accepted 12 April 1999

Abstract

α,β-Poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA), a synthetic water-soluble biocompatible polymer, was derivatized with glycidyl methacrylate (GMA), in order to introduce in its structure chemical residues having double bonds and ester groups. The obtained copolymer (PHG) contained 29 mol% of GMA residues. PHG aqueous solutions at various concentrations ranging from 30 to 70 mg/ml were exposed to a source of UV rays at λ 254 nm in the presence or in the absence of *N*,*N*′-methylenebisacrylamide (BIS); the formation of compact gel phases was observed beginning from 50 mg/ml. The obtained networks were characterized by FT-IR spectrophotometry and swelling measurements which evidenced the high affinity of PHG hydrogels towards aqueous media at different pH values. In vitro chemical or enzymatic hydrolysis studies suggested that the prepared samples undergo a partial degradation both at pH 1 and pH 10 and after incubation with enzymes such as esterase, pepsin and α-chymotrypsin. Finally, the effect of irradiation time on the yield and the properties of these hydrogels was investigated and the sol fractions coming from irradiated samples, properly purified, were characterized by FT-IR and ^{1}H-NMR analyses. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: α,β-Poly(*N*-2-hydroxyethyl)-DL-aspartamide; Glycidyl methacrylate; Ultraviolet irradiation; Hydrogel; Biodegradability

1. Introduction

In the area of controlled drug delivery much attention has been recently directed to biodegradable hydrogels [1–7]. They can be prepared from water-soluble polymers which crosslink either by chemical reaction with or without crosslinking agents or by irradiation by using UV, γ- or β-rays [8–12]. In this context our interest is addressed to the preparation of hydrogels employing as starting polymer a water-soluble macromolecule at protein-like structure, i.e. α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) [13] and UV irradiation as preparation procedure.

Already for several years our research group has been working on a thorough investigation of PHEA [14–17] since the favorable toxicological and physico-chemical properties of this polymer allow it to be proposed as carrier in macromolecular prodrugs [18–21] and starting material to prepare hydrogels by γ-irradiation [22–24].

Now our attention is focused on the preparation of hydrogels using UV rays, since they give several

* Corresponding author. Fax: +39 (91) 6177333; E-mail: gaegiamm@mbox.unipa.it

0304-4165/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0304-4165(99)00051-3

INSDOCID: <XP_____4276327A__1_>

advantages in comparison with γ-rays. In particular, γ-rays have a higher energy and give rise to the breaking of chemical bonds in a non-selective and difficult to check way. In effect, besides homolytic breaking of π bonds, γ-irradiation can give rise to breaking of σ bonds, which are not involved in the crosslinking process, thus causing degradation phenomena in the irradiated polymer. UV irradiation is more selective and involves only the chromophores able to absorb radiations with a suitable energy. The portion of a polymer which is not photochemically active does not interact with UV rays and therefore does not undergo any primary photochemical effect [25]. However, relatively little work has been reported on the preparation of hydrogels via photocrosslinking of water soluble polymers for all the advantages of this procedure [26–28].

In this context, during preliminary experiments PHEA was subjected to the action of UV rays both at solid state and in aqueous solution at different concentrations, but it did not show any chemical alteration. Then, in order to increase the reactivity of this polymer towards UV rays, we properly derivatized PHEA introducing into its side chains specific chromophore groups. To this aim, we derivatized PHEA with glycidyl methacrylate (GMA) which contains in the same molecule an ester group conjugated to a double bond. Besides increasing the reactivity of PHEA towards UV activated reactions, this approach should allow us to obtain potentially biodegradable hydrogels provided that ester groups remain after the crosslinking process.

In a previous article a series of derivatized PHEA samples at various GMA content (PHG copolymers) had been obtained by varying some reaction parameters as solvent, catalyst, pH, GMA concentration and reaction time [29].

In the present paper we report the formation of new hydrogels obtained by exposing aqueous solutions of a PHG sample with a derivatization degree of 29 mol% to UV rays in the presence or in the absence of *N,N'*-methylenebisacrylamide (BIS). Hydrogels have been characterized by FT-IR spectrophotometry and swelling measurements in aqueous media at different pH values. In vitro studies of chemical and enzymatic hydrolysis on the obtained networks have been carried out to confirm their suggested biodegradability. The effect of irradiation time on yield and properties of PHG hydrogels has been also investigated. Finally, in order to obtain further information on the processes which occur during UV irradiation in the adopted experimental conditions, the sol fractions have been purified and characterized by FT-IR and ^{1}H-NMR analyses.

2. Materials and methods

2.1. Chemicals

All used reagents were of analytical grade, unless otherwise stated. D,L-Aspartic acid, ethanolamine, *N,N*-dimethylformamide (DMF), anhydrous *N,N*-dimethylacetamide (DMA) and BIS were from Fluka (Switzerland). GMA, 4-dimethylaminopyridine (4-DMAP) and D_2O (isotopic purity 99.9%) were purchased from Aldrich Chemical (St. Louis, MO, USA). Pepsin from porcine stomach mucosa, 3500 U (mg protein)$^{-1}$ and α-chymotrypsin from bovine pancreas, 49 U (mg protein)$^{-1}$ were purchased from Sigma (USA). Esterase from hog liver, 130 U (mg protein)$^{-1}$, was obtained from Boehringer Mannheim (Germany).

2.2. Apparatus

Molecular weights and polydispersity indices of starting PHEA and PHG copolymer were determined by light scattering measurements, using a Dawn DSP-F Laser Spectra Physics Spectrometer.

Elemental analyses (C, H, N) were carried out on a Carlo Erba model 1106 analyzer; PHG was quantitatively dried before analysis under reduced pressure (10^{-3} mm Hg) at room temperature for 48 h on P_2O_5.

^{1}H-NMR spectra were obtained with a Bruker AC-250 instrument operating at 250.13 MHz.

FT-IR spectra were recorded using a FT-IR Vector 22 Bruker spectrometer as pellets in KBr in the range 4000–400 cm^{-1}. The resolution was 1 cm^{-1}. The deuterated spectra were obtained on samples solubilized or dispersed in D_2O and then dried.

Viscosity measurements were carried out at 25 ± 0.01°C using an Ubbelohde microviscometer equipped with an AVS 440 automatic viscosity measuring unit from Schott.

NSDOCID: <XP_____4276327A_I_>

UV spectra were obtained on a Perkin-Elmer 330 instrument equipped with a 3600 data station.

UV irradiation was performed using a Rayonet reactor equipped with a Rayonet Carousel Motor Assembly and 16 mercury lamps of 8 W at low pressure with an emission at λ 254 nm.

Centrifugation was performed with an International Equipment Company Centra MP4R equipped with a 854 rotor and temperature control.

2.3. PHEA synthesis

PHEA was prepared by reaction of a polysuccinimide (PSI), obtained by thermal polycondensation of D,L-aspartic acid, with ethanolamine in DMF solution and purified according to a elsewhere reported procedure [13]. Spectroscopic data (FT-IR and ^{1}H-NMR) were in agreement with the literature values [19]. The batch of PHEA used in the present study had a weight-average molecular weight of 56900 ($M_w/M_n = 1.79$); $[\eta] = 25.6$ ml/g.

2.4. Derivatization of PHEA with glycidyl methacrylate (PHG copolymer)

The reaction between PHEA and GMA was carried out in organic phase using a procedure reported elsewhere [29]. The degree of derivatization (DD) of prepared PHG was determined by ^{1}H-NMR and resulted to be 29 mol%. PHG copolymer was also characterized by FT-IR spectrophotometry and light scattering measurements, ($M_w = 70000$, $M_w/M_n = 1.84$); $[\eta] = 26.2$ ml/g.

2.5. Viscosity measurements

Intrinsic viscosity, $[\eta]$, of PHEA or PHG was determined in distilled water measuring reduced viscosities in the range 2–10 mg/ml and extrapolating the concentration to zero.

2.6. UV irradiation of PHEA

PHEA at the solid state or in aqueous solution (concentration range 20–100 mg/ml) was placed in quartz tubes each equipped with an internal quartz piston in order to have about 2 mm in thickness of sample, then irradiated under nitrogen at λ 254 nm for 10 h. No appearance of insoluble network was observed. Then, the sample was dialyzed utilizing Visking dialysis tubing (18/32 inch) with a molecular weight cut-off of 12000–14000. After dialysis, the polymer solution was concentrated under vacuum and lyophilized. The obtained product was weighed and characterized by FT-IR spectrophotometry, ^{1}H-NMR analysis and viscosity measurements.

2.7. Formation of polymeric networks by UV irradiation of PHG

Aqueous solutions of PHG copolymer (concentration range 30–70 mg/ml) with or without BIS (25 mol% with respect to the mols of GMA linked to PHEA) were placed in quartz tubes (see above) then irradiated for 3.5 h under nitrogen at λ 254 nm. After this time polymer solutions were observed and the samples producing an insoluble network were removed. Every gel was purified by several washes with distilled water, centrifuged at 12000 rpm at 4°C for 20 min then lyophilized, weighed and characterized by FT-IR spectrophotometry.

2.8. Study of sol fractions

The liquid aqueous phase which separates from gel phase during irradiation process performed on a PHG solution (60 mg/ml) both in the presence or in the absence of BIS, was added to the washing liquids (see Section 2.7) of corresponding gel phase and lyophilized. The obtained product was dissolved in distilled water and subjected to extensive dialysis utilizing Visking dialysis tubing (18/32 inch) with a molecular weight cut-off of 12000–14000. After dialysis the solution was lyophilized and the obtained product was characterized by FT-IR and ^{1}H-NMR analyses.

^{1}H-NMR (D_2O) spectra still showed the presence of signals at low intensity, due to residues of GMA linked to PHEA: δ 1.94 [s, 3H, -CO-C(**CH_3**) = CH_2], 4.28 [m, 1H, -O-CH_2-**CH**(OH)-CH_2-], 5.75 and 6.15 [2s, 2H, -CO-C(CH_3) = **CH_2**].

2.9. Swelling studies

Swelling studies were performed on the samples obtained by the irradiation of 60 mg/ml PHG solu-

)NSDOCID: <XP_____4276327A__I_>

tions with and without BIS (samples PHG-UV rays and (PHG+BIS)-UV rays, respectively). Swelling ability of these samples was determined at 25°C in double-distilled water, HCl 0.1 N (pH 1) and phosphate buffers (NaCl, Na_2HPO_4, KH_2PO_4) at pH 6.8 and 7.4. In particular, aliquots of each dried and exactly weighed sample were kept in contact with the penetrant medium until the equilibrium swelling was reached then, each swollen sample was filtered, plugged by blotting paper and weighed. Water content (WC %) was calculated as follows:

$$WC(\%) = (W_s - W_d)/W_s \times 100$$

where W_s and W_d are the weight of swollen and dry sample respectively.

2.10. Chemical hydrolysis of PHG-UV rays and (PHG+BIS)-UV rays hydrogels

In vitro stability of PHG-UV rays and (PHG+BIS)-UV rays hydrogels was investigated in HCl 0.1 N (pH 1), phosphate buffer solution at pH 7.4 (NaCl, Na_2HPO_4, KH_2PO_4) and NaOH 10^{-4} N (pH 10). Samples (25 mg) were dispersed in 10 ml of liquid medium, then kept in a water bath at 37±0.1°C with continuous stirring (100 rpm) for 5 days. After this time, samples were neutralized, centrifuged at 12000 rpm at 10°C for 15 min and the supernatant was separated. For each sample, the solid precipitate was washed five times with distilled water under continuous stirring at 37°C for 1 h to extract any soluble polymer chain and electrolytes entrapped in the network. Finally, each sample was washed with acetone and centrifuged at 12000 rpm at 10°C for 15 min. The recovered solid residue was then dried, weighed and characterized by FT-IR analysis and swelling studies in double-distilled water. Each experiment was performed in triplicate and results were in agreement within ±2% error.

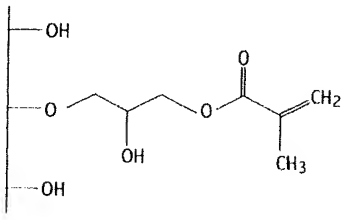
2.11. Enzymatic hydrolysis of PHG-UV rays and (PHG+BIS)-UV rays hydrogels

Aliquots (25 mg) of PHG-UV rays and (PHG+BIS)-UV rays hydrogels were incubated under continuous stirring (100 rpm) at 37±0.1°C by using a water bath with: (a) 2 ml HCl solution (0.01 N) at pH 2 containing pepsin (final enzyme concentration 0.8 mg/ml) for 24 h; (b) 2 ml of TRIS buffer solution at pH 8 containing $CaCl_2$ 0.1 M and α-chymotrypsin (final enzyme concentration 0.8 mg/ml) for 24 h; (c) 2 ml of phosphate buffer solution (NaCl, Na_2HPO_4, KH_2PO_4) containing esterase (final enzyme concentration 0.9 mg/ml) for 5 days. Enzyme solutions were prepared immediately before the experiment. After each experiment, samples were neutralized (when necessary), purified and characterized by the same methods used for samples obtained after chemical hydrolysis.

3. Results and discussion

The use of UV rays in order to prepare crosslinked polymer systems is a very profitable method due to its easiness of approach and the possibility to obtain pure products, i.e. not contaminated with residuals of toxic initiators or crosslinking agents [30,31]. In this context, PHEA has been irradiated at λ 254 nm both in the solid state and in aqueous solution at various concentrations, but as expected, this polymer does not undergo any photolytic effect or crosslinking process. In effect, the only chromophores of PHEA are the amide groups of main backbone which do not show a valuable absorption at λ 254 nm. Then, after UV irradiation, the polymer was purified by dialysis, lyophilized, weighed and characterized. No variation in the weight, spectral data (FT-IR, ^{1}H-NMR) and [η] value was found in comparison with starting PHEA.

However, it is known that a partial chemical modification of a macromolecule is a strategy pursued in order to improve its reactivity towards a particular reaction, such as crosslinking, grafting or linkage with a biologically active agent. Then, in order to prepare new crosslinked materials through UV irra-



PHG

Fig. 1. Chemical structure of PHG copolymer.

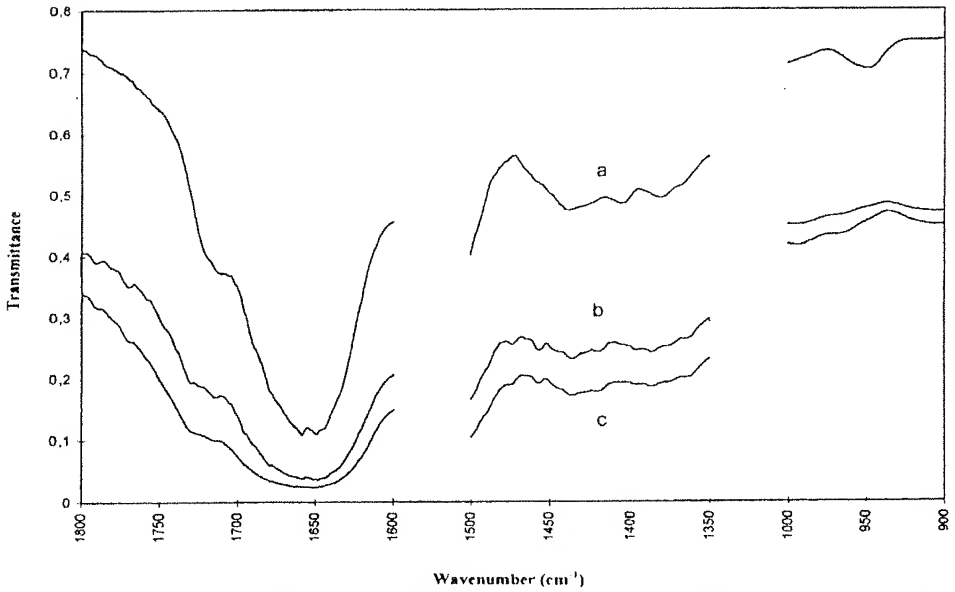


Fig. 2. FT-IR spectra of PHG (a), PHG-UV ray (b) and (PHG+BIS)-UV ray (c) samples.

diation we first acryloylated PHEA which was subsequently subjected to UV ray action.

In particular, the reaction between GMA and PHEA gives rise to the formation of PHG copolymer shown in Fig. 1. PHG copolymer which was water-soluble, like starting PHEA, was characterized by FT-IR spectroscopy and ^{1}H-NMR analysis.

In particular, FT-IR spectrum of PHG, compared to PHEA one, shows new bands due to the introduction of GMA residues. As reported in Fig. 2 (spectrum a) the most important signals are: (i) the ester band of GMA unit shifted to lower frequency (1720 cm^{-1}) because this group is involved in hydrogen bond and conjugated with the double bond; (ii) the two medium-weak bands of the vinylidene CH deformation at 1405 and 950 cm^{-1} due to scissoring and wagging moves, respectively. The degree of derivatization determined by ^{1}H-NMR analysis was equal to 29 mol%. The UV spectrum of PHG aqueous solution shows an absorption band within the range 240–330 nm in which the starting polymer does not absorb (see Fig. 3).

3.1. Formation of polymeric networks by UV irradiation of PHG and their characterization

Aqueous solutions of PHG copolymer (range 30–70 mg/ml) in the presence and in the absence of a biodegradable crosslinking agent, such as BIS [32] (25 mol% with respect to the mols of GMA linked to PHEA), were irradiated under nitrogen for 3.5 h at λ 254 nm and the obtained gels were purified as described in Section 2. The percentage (w/w) of gel phase based on the starting PHG versus PHG concentration in the presence or in the absence of BIS is reported in Fig. 4.

In effect the smallest concentration of PHG which gave rise to an insoluble product was 50 mg/ml. The yield of gel phase increases by increasing PHG concentration and, as expected, the presence of BIS causes an increase in the weight of gel phase due to the participation of this agent in the crosslinking

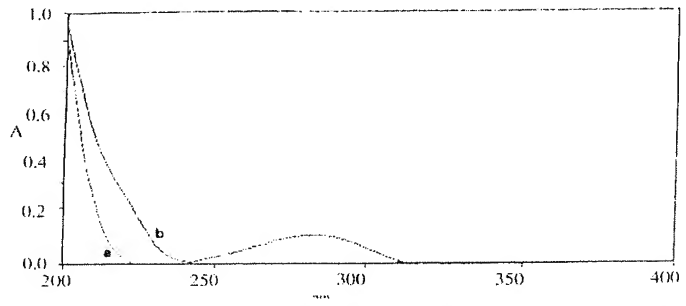


Fig. 3. UV spectra of PHEA (a) and PHG (b) aqueous solutions.

3NSDOCID: <XP_____4276327A_I_>

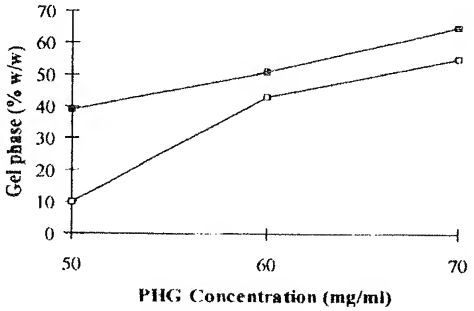


Fig. 4. Percentage of gel phase versus PHG concentration in the presence (■) and in the absence (□) of BIS.

process. All obtained networks were insoluble in water and in the common organic solvents, such as dichloromethane, acetone, ethanol, dimethylsulfoxide, dimethylacetamide, dimethylformamide.

FT-IR spectra of PHG hydrogels obtained in the presence or in the absence of BIS show different peaks in comparison with starting uncrosslinked PHG (see Fig. 2, spectra b and c compared with spectrum a). The more interesting feature is the shift to higher frequency (1730 cm^{-1}) of ester group asymmetric stretching. This shift suggests the lack of conjugation with the double bond in the GMA residue, which bands (at 1405 and 950 cm^{-1}), furthermore, are not visible in the spectra. This points to the opening of the double bond caused by UV irradiation and the occurrence of inter- and intra-polymeric chain crosslinking.

In order to evaluate the affinity of prepared networks towards aqueous medium, swelling studies have been carried out at 37°C on dried PHG-UV rays and (PHG+BIS)-UV rays samples in the presence of various media mimicking physiological environments such as solutions at pH 1 (simulated gastric fluid), pH 6.8 (simulated intestinal fluid) and pH 7.4 (simulated extracellular fluid).

Table 1
Swelling ability of PHG-UV ray and (PHG+BIS)-UV ray samples in various media

	Water content (%)			
	H_2O	pH 1	pH 6.8	pH 7.4
PHG-UV rays	88.4	58.6	87.1	88.1
(PHG+BIS)-UV rays	88.9	63.5	88.5	85.7

The values of water content (WC %) for the investigated samples are reported in Table 1.

In particular, for the investigated samples, the swelling degree does not change significantly in media with a pH value close to neutrality (double-distilled water, buffers at pH 6.8 and 7.4). The observed small differences could be related to the different osmotic pressure and ionic strength of the swelling medium. In contrast, the swelling at acidic pH is lower than that found in other media, similar to PHG hydrogels obtained through γ-irradiation [33]. In addition, the presence of BIS affects slightly the swelling of the prepared networks. However, the values of water content reported in Table 1 show the outstanding swelling of PHG-UV rays and (PHG+BIS)-UV rays samples. This result is an essential property of hydrogel systems because it grants them a potential biocompatibility and affects the ability to release en-

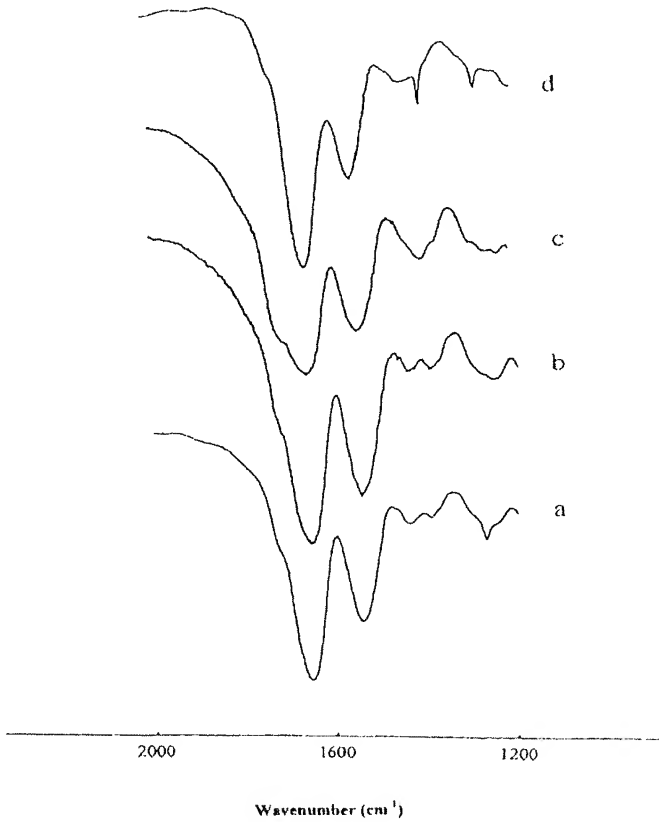


Fig. 5. FT-IR spectra of PHG-UV ray sample before chemical treatment (a) and after treatment at pH 7.4 (b), pH 1 (c) and pH 10 (d).

Table 2
Yield (%) after chemical or enzymatic hydrolysis of PHG-UV ray and (PHG+BIS)-UV ray samples

	Yield (%) after chemical or enzymatic hydrolysis	
	PHG-UV rays	(PHG+BIS)-UV rays
Sample incubated at pH 1	89	91
Sample incubated at pH 7.4	96	97
Sample incubated at pH 10	70	76
Sample treated with esterase	73	73
Sample treated with pepsin	93	95
Sample treated with α-chymotrypsin	97	98

trapped solutes, such as drug or enzyme molecules, in a physiological medium.

Besides, the presence of ester groups in the chemical structure of both PHG-UV rays and (PHG+BIS)-UV rays networks confers a potential biodegradability to these systems, so we considered it interesting to evaluate their stability after chemical or enzymatic treatments.

The chemical stability of PHG-UV rays and (PHG+BIS)-UV rays samples was evaluated by incubating them in HCl (pH 1), phosphate buffer (pH 7.4) and NaOH (pH 10) solutions at 37°C for 5 days.

The yield of samples recovered after this treatment is reported in Table 2. The values of WC %, determined in double-distilled water, of the samples recovered after hydrolytic treatment are reported in Table 3.

The increase of swelling of the samples after hydrolytic treatment at pH 1 and pH 10 suggests that the prepared hydrogels undergo a partial degradation in these media. This result has been also confirmed by FT-IR spectra (see Fig. 5). In particular, at pH 7.4 the intensities and the positions of the bands remain unaffected after the treatment. At pH 1 just an increase in intensity and a shift to lower frequencies of the ester CO asymmetric stretching were evidenced, due to a partial hydrolysis of the ester group with formation of alcoholic and acid residues along the polymeric matrix. At pH 10 an impressive reduction of ester asymmetric stretching was recorded because of hydrolysis and formation of carboxylate and alcoholic groups. Similar results have been obtained for the (PHG+BIS)-UV rays sample.

In order to evaluate the stability of PHG-UV ray and (PHG+BIS)-UV ray samples towards the enzymes of the gastrointestinal tract, hydrolysis assays have been performed in the presence of pepsin and α-chymotrypsin. Due to the presence of ester bonds, hydrolytic treatment was also carried out in the presence of esterase.

In particular, PHG-UV ray and (PHG+BIS)-UV ray samples were incubated at 37°C in the presence of pepsin or α-chymotrypsin for 24 h and in the presence of esterase for 5 days. The yield of samples recovered after enzymatic treatment is also reported in Table 2.

Table 3
Swelling ability (determined in double-distilled water) after chemical or enzymatic hydrolysis of PHG-UV ray and (PHG+BIS)-UV ray samples

	Water content (%) after chemical or enzymatic hydrolysis	
	PHG-UV rays	(PHG+BIS)-UV rays
Sample not treated	76.8	80.6
Sample incubated at pH 1	85.2	87.6
Sample incubated at pH 7.4	77.3	81.2
Sample incubated at pH 10	88.3	92.1
Sample treated with esterase	96.1	95.5
Sample treated with pepsin	91.3	94.1
Sample treated with α-chymotrypsin	91.2	92.6

\SDOCID: <XP_____4276327A__I_>

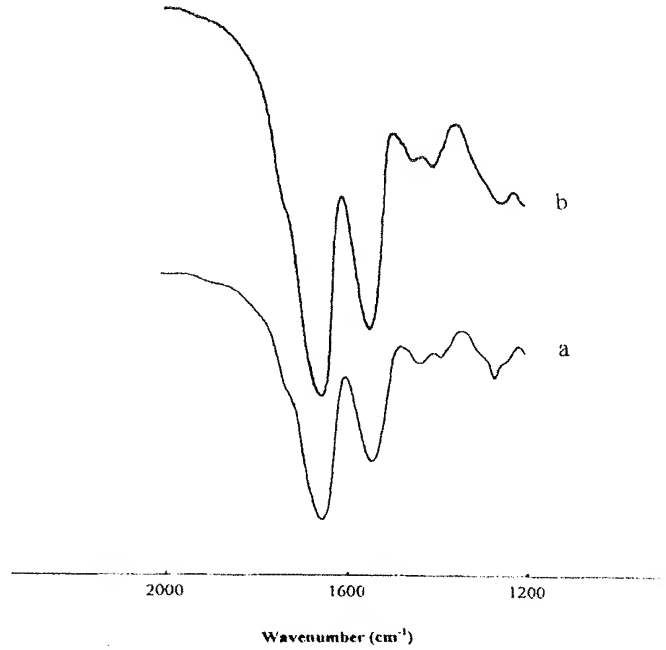


Fig. 6. FT-IR spectra of PHG-UV ray sample before (a) and after (b) incubation with esterase.

As far as FT-IR analysis is concerned, only the samples treated with esterase showed any modification in the spectrum (see Fig. 6), where the intensity of ester band was reduced. This result suggests a slow action of the enzyme preferentially towards external sites of the crosslinked polymeric matrix. Similar results have been obtained for the (PHG+BIS)-UV ray sample. The values of WC % (determined in double-distilled water) of the samples recovered after enzymatic treatment are also reported in Table 3.

The increase of swelling of the samples after enzymatic treatment suggests that the prepared hydrogels undergo a decrease of the crosslinking degree, i.e. a partial degradation.

Table 4
Effect of irradiation time on yield (%) of PHG-UV ray and (PHG+BIS)-UV ray samples

Yield (%)		
Irradiation time (h)	PHG-UV rays	(PHG+BIS)-UV rays
1	51	53
2	46	55
3.5	43	51
7	38	45

3.2. Influence of irradiation time

In order to evaluate the effect of irradiation time on yield % of prepared hydrogels, aqueous solutions of PHG (60 mg/ml) were irradiated at 254 nm for 1, 2 and 7 h besides 3.5 h in the presence or in the absence of BIS. Small differences of yield % as a function of irradiation time have been found, as evidenced in Table 4. Besides all samples obtained at different irradiation times have been characterized by swelling measurements in double-distilled water and at pH 1 (see Table 5) and FT-IR analysis.

The increase of WC % of samples irradiated for 1 and 2 h, in comparison with the samples irradiated for 3.5 h, could be due to an incomplete crosslinking of PHG chains. FT-IR spectra are in agreement with this supposition since the bands of unsaturations of GMA residues linked to PHG (and BIS molecules if used) are still present even if with a low intensity.

Instead, the increase of swelling degree of the samples irradiated for 7 h and the lower yield % value in comparison with the samples irradiated for 3.5 h could be due to probable degradation phenomena which occur on side chains of polymeric material in consequence of a prolonged exposure to UV rays.

However, FT-IR spectra of the samples irradiated for 7 h do not show valuable differences in comparison with that ones irradiated for 3.5 h.

3.3. Study of sol fractions

Finally, in order to have a greater knowledge of the processes occurring during UV irradiation in the used experimental conditions, the sol fractions related to PHG-UV rays and (PHG+BIS)-UV rays samples were dialyzed (see Section 2) and recovered

Table 5
Effect of irradiation time on swelling ability of PHG-UV ray and (PHG+BIS)-UV ray samples

Irradiation time (h)	Water content (%)			
	PHG-UV rays		(PHG+BIS)-UV rays	
	H_2O	pH 1	H_2O	pH 1
1	93.9	80.9	87.3	81.3
2	84.2	74.4	84.5	72.4
3.5	76.8	58.6	80.6	63.5
7	86.3	75.1	84.9	69.2

NSDOCID: <XP_____4276327A__I_>

after lyophilization. The yield of sol fractions recovered after dialysis was 8.75 and 5.5% w/w, based on starting PHG, for samples corresponding to PHG-UV rays and (PHG+BIS)-UV rays, respectively. Besides, the yield of dialyzed sol fractions (15.9% and 11.6% based on sol fractions before purification) suggests that during irradiation process probably degradation phenomena occur on the main backbone and side chains of PHG producing chains at low molecular weight which are removed by dialysis.

Besides, both purified sol fractions have been characterized by FT-IR and ^{1}H-NMR analyses. In particular, the FT-IR spectrum of the purified PHG-UV ray sol fraction showed a drastic lowering of the ester band probably due to breaking of side chains of polymer.

The structural variations seem point to a 'relaxation' of the polymer with a lowering of hydrogen bonds as suggested by a narrow band centered at 3420 cm^{-1}, by the presence of shoulders to higher frequencies, CO of secondary alcohol, over the primary alcohol band.

^{1}H-NMR spectra show also a decrease of integral values related to GMA residues which remain linked to uncrosslinked polymer fraction (about 2.5 and 6.12 mol% for the PHG-UV ray and (PHG+BIS)-UV ray sol fractions respectively). This result, in agreement with FT-IR analysis, confirms the occurrence of degradation phenomena on PHG side chains.

4. Conclusions

The derivatization of PHEA with GMA is a profitable method to introduce pendant double bonds and ester groups in side chains of this macromolecule. The presence of GMA residues makes starting polymer properly reactive towards the reactions activated by UV rays in order to obtain crosslinked structures. In fact, the exposure of aqueous solutions of the functionalized polymer (PHG) at a wavelength of 254 nm in the presence or in the absence of a biodegradable crosslinker such as BIS gives rise to the formation of hydrogels exhibiting a high affinity towards aqueous media mimicking biological fluids. In addition, due to the presence of ester groups which are maintained after crosslinking process these hydrogels undergo a partial degradation by chemical or enzymatic hydrolysis.

Acknowledgements

This work was financially supported by CNR and MURST grants.

References

[1] K. Kamath, K. Park, Biodegradable hydrogels in drug delivery, Adv. Drug Delivery Rev. 11 (1993) 59–84.
[2] S. Dumitriu, C. Dumitriu-Medvichi, Hydrogel and General Properties of biomaterials in: S. Dumitriu (Ed.), Polymeric Biomaterials, Marcel Dekker, New York, 1994, pp. 3–97.
[3] J. Heller, Biodegradable hydrogels, in: N.A. Peppas (Ed.), Hydrogels in Medicine and Pharmacy, Vol. III: Properties and Applications, CRC Press, Boca Raton, FL, 1987, pp. 137–149.
[4] N.R. Vyavahare, M.G. Kulkarni, R.A. Mashelkar, Matrix systems for zero-order release: facile erosion of crosslinked hydrogels, Polymer 33 (1992) 593–599.
[5] H. Brondsted, J. Kopecek, Hydrogels for site-specific oral drug delivery: synthesis and characterization, Biomaterials 12 (1991) 584–592.
[6] V. Ranade, Drug Delivery Systems, 5 A: Oral drug delivery, J. Chim. Pharmacol. 32 (1991) 2–16.
[7] M. Singh, R. Rathi, A. Singh, J. Heller, G.P. Talwar, J. Kopecek, Controlled release of LHRH-DT from bioerodible hydrogel microspheres, Int. J. Pharmacol. 76 (1991) R5–R8.
[8] K. Park, Enzyme-digestible swelling hydrogels as platforms for long-term oral drug delivery: synthesis and characterization, Biomaterials 9 (1988) 435–441.
[9] K. Ulbrich, V. Subr, L.W. Seymour, R. Duncan, Novel biodegradable hydrogels prepared using the divinylic cross-linking agent N,O-dimethacryloylhydroxylamine. I. Synthesis and characterization of rates of gel degradation and rate of release of model drugs, in vitro and in vivo, J. Control. Release 24 (1993) 181–190.
[10] N. Yui, T. Okano, Y. Sakurai, Inflammation responsive degradation of crosslinked hyaluronic acid gels, J. Control. Release 22 (1992) 105–116.
[11] A.S. Hoffman, A review of the radiation use plus chemical and biochemical processing treatments to prepare novel biomaterials, Radiat. Phys. Chem. 18 (1981) 323–342.
[12] A. Muhlebach, B. Muller, C. Pharisa, M. Hofmann, B. Seiferling, D. Guerry, New water-soluble photo crosslinkable polymers based on modified poly(vinyl alcohol), J. Polymer Sci. Part A Polymer Chem. 35 (1997) 3603–3609.
[13] G. Giammona, B. Carlisi, S. Palazzo, Reaction of α,β-poly-(*N*-hydroxyethyl)-DL-aspartamide with derivatives of carboxylic acids, J. Polymer Chem. 40 (1987) 2813–2818.

INSDOCID: <XP_____4276327A__I_ >

[14] G. Giammona, B. Carlisi, G. Cavallaro, I. Donato, F. Pinio, V. Turco Liveri, Calorimetric investigation of the interaction between α,β-poly(*N*-hydroxyethyl)-DL-aspartamide and surfactants, Int. J. Pharmacol. 64 (1990) 239–242.

[15] G. Cavallaro, G. Giammona, G. La Manna, S. Palazzo, G. Pitarresi, V. Turco Liveri, Viscosimetric investigation of the interaction between sodium dodecylsulfate micelles and a polymer drug carrier, Int. J. Pharmacol. 90 (1993) 195–201.

[16] G. Cavallaro, G. Giammona, M. Goffredi, G. La Manna, G. Pitarresi, V. Turco Liveri, Dielectric behavior of aqueous solutions of α,β-poly(asparthyl hydrazide) and α,β-poly-(*N*-hydroxyethyl)-DL-aspartamide: an investigation of the structural and dynamical properties, J. Bioact. Compat. Polymers 9 (1994) 101–110.

[17] T. Coviello, Y. Yuguchi, K. Kajiwara, G. Giammona, G. Cavallaro, F. Alhaique, A. Palleschi, Conformational analysis of α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) and α,β-polyasparthydrazide (PAHy) polymers in aqueous solution, Polymer 39 (1998) 4159–4164.

[18] G. Giammona, B. Carlisi, G. Pitarresi, G. Fontana, Hydrophilic and hydrophobic polymeric derivatives of anti-inflammatory agents such as alclofenac, ketoprofen and ibuprofen, J. Bioact. Compat. Polymers 6 (1991) 129–141.

[19] G. Giammona, G. Puglisi, G. Cavallaro, A. Spadaro, G. Pitarresi, Chemical stability and bioavailability of acyclovir coupled to α,β-poly(*N*-hydroxyethyl)-DL-aspartamide, J. Control. Release 33 (1995) 261–271.

[20] G. Giammona, G. Cavallaro, G. Fontana, G. De Guidi, S. Giuffrida, Macromolecular prodrug of diflunisal. II. Investigations of in vitro release and of photochemical behaviour, Eur. J. Pharmacol. Sci. 4 (1996) 273–282.

[21] G. Giammona, G. Cavallaro, G. Pitarresi, G. Fontana, B. Carlisi, Coupling of the antiviral agent zidovudine to polyaspartamide and in vitro drug release studies, J. Control. Release 54 (1998) 321–331.

[22] G. Spadaro, C. Dispenza, G. Giammona, G. Pitarresi, G. Cavallaro, Cytarabine release from α,β-poly(*N*-hydroxyethyl)-DL-aspartamide matrices cross-linked through gamma radiation, Biomaterials 17 (1996) 953–958.

[23] F. Castelli, G. Pitarresi, V. Tomarchio, G. Giammona, Effect of pH on the transfer kinetics of an anti-inflammatory drug from polyaspartamide hydrogels to a lipid model membrane, J. Control. Release 45 (1997) 103–111.

[24] G. Giammona, G. Pitarresi, V. Tomarchio, S. Cacciaguerra, P. Govoni, A hydrogel based on a polyaspartamide: characterization and evaluation of in vivo-biocompatibility and drug release in the rat, J. Pharm. Pharmacol. 49 (1997) 1051–1056.

[25] N.J. Turro, in: Modern Molecular Photochemistry, Benjamin/Cummings, Menlo Park, CA, 1978.

[26] A.S. Sawhney, C.P. Pathak, J.A. Hubbell, Bioerodible hydrogels based on photopolymerized poly(ethylene glycol)-co-poly(α-hydroxy acid) diacrylate macromers, Macromolecules 26 (1993) 581–587.

[27] M. Doytcheva, D. Dotcheva, R. Stamenova, A. Orahovats, Ch. Tsvetanov, J. Leder, Ultraviolet-induced crosslinking of solid poly(ethylene oxide), J. Appl. Polymer Sci. 64 (1997) 2299–2307.

[28] N. Vyavahare, J. Kohn, Photocrosslinked hydrogels based on copolymers of poly(ethylene glycol) and lysine, J. Polymer Sci. Part A Polymer Chem. 32 (1994) 1271–1281.

[29] G. Giammona, V. Tomarchio, G. Pitarresi, G. Cavallaro, Glycidyl methacrylate derivatization of α,β-poly(*N*-hydroxyethyl)-DL-aspartamide and α,β-polyasparthydrazide, Polymer 38 (1997) 3315–3323.

[30] F.M. Andreopoulos, C.R. Deible, M.T. Stauffer, S.G. Weber, W.R. Wagner, E.J. Beckman, A.J. Russel, Photoscissable hydrogel synthesis via rapid photopolymerization of novel PEG-based polymers in the absence of photoinitiators, J. Am. Chem. Soc. 118 (1996) 6235–6240.

[31] Y. Nakayama, T. Matsuda, Preparation and characteristics of photocrosslinkable hydrophilic polymer having cinnamate moiety, J. Polymer Sci. Part A Polymer Chem. 30 (1992) 2451–2457.

[32] V.P. Torchilin, E.G. Tidchenko, V.N. Smirnov, E.I. Charov, Immobilization of enzymes on slowly soluble carriers, J. Biomed. Mater. Res. 11 (1977) 223–235.

[33] G. Giammona, G. Pitarresi, G. Cavallaro, G. Spadaro, New biodegradable hydrogels based on an acryloylated polyaspartamide crosslinked by gamma irradiation, J. Biomater. Sci. Polymer (submitted).

NSDOCID: <XP_____4276327A__I_>